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Award Number: W81XWH-06-1-0312

TITLE: An epidemiologic study of genetic variation in hormonal pathways in relation to the effect of hormone replacement therapy on breast cancer risk

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REPORT DATE: April 2007

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
01/04/07	Annual	15 Mar 2006 – 14 Mar 2007

4. TITLE AND SUBTITLE

An epidemiologic study of genetic variation in hormonal pathways in relation to the effect of hormone replacement therapy on breast cancer risk

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-06-1-0312

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Fred Hutchinson Cancer Research Center
Seattle, WA 98109-1024

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT: Genetic variation in the catechol estrogen (CE) metabolism pathway may modify the effect of combine hormone therapy (CHT). In a population-based case-control study of breast cancer in women aged 65-79, 891 cases and 878 controls were genotyped for functional single nucleotide polymorphisms (SNPs) in the CYP1B1, COMT, GSTT1, GSTM1, and GSTP1 genes. Women who carried at least one copy of the A allele in the GSTP1 gene (105 Ile; rs1695) had a 1.4 -fold increased risk of breast cancer compared to those who were homozygous for the G allele (95% Confidence Interval (CI) 1.1-1.9); women homozygous with the T allele in the CYP1B1*2 gene (119 Ser; rs1056827) were at 1.8 (95% CI:1.2-2.6) times the risk of those carrying at least one copy of the G allele; no other single genes demonstrated significant associations nor did those single genes have a significant interaction with CHT. In a multi-gene model limited to genes with single gene effects (CYP1B1*2 and GSTP1), the risk of breast cancer increased as the number of high risk genotypes increased (OR =1.6 [95% CI: 1.01-2.3] for 1 vs. 0 high risk genotypes; OR = 2.8 [95% CI:1.5-5.2] 2 vs. 0 high risk genotypes). This association was heightened among current, long-term (60+ months) CHT users, (OR = 7.4 [95% CI 1.9-28.1] for 1-2 vs. 0 high risk genotypes), while in non-users of CHT, the association was attenuated (OR = 1.3 [95% CI 0.8-2.1] for 1-2 vs. 0 high risk genotypes). These results suggest the risk of breast cancer among CHT users is modified by genetic variation in the catechol estrogen metabolism pathway.

15. SUBJECT TERMS
Genetic polymorphisms, exogenous risk factors, gene-environment interaction, hormonal pathway, epidemiology, estrogen, progesterone, tagSNP

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
U	U	U	UU	8	**19b. TELEPHONE NUMBER** *(include area code)*

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Introduction

This document describes the work completed during the first year of the pre-doctoral training grant for the Breast Cancer Research Program. Overall, much has been accomplished and I have made great progress towards graduation. I am on track with the timeline set out in the statement of purpose and in some cases am ahead of schedule. This annual report documents the work completed in year one, by listing all the work that has been completed and describing any modifications to the tasks set forth in the application.

Body

The work completed to date has included the completion of lab sample preparation, coursework, manuscript preparation, and completion of the doctoral general exam. In year 1 of the funding period, all work described in the statement of work for months 1-12 has been completed (with the exception of one academic course which was not offered by the University of Washington during the year). For a comprehensive list of tasks completed, please refer to Table 1 in the Appendix. Furthermore, I have begun making progress on tasks listed to be completed during year 2 of the training grant and am on track to graduate as set out in the statement of work.

Some highlights from the completed tasks include progress on data analysis for part 1 of my dissertation project. A poster emanating from this work, entitled 'Genetic polymorphisms in the estrogen metabolism pathway as modifiers of the effect of hormone therapy in breast cancer risk' has been accepted for presentation at the American Association of Cancer Researchers (AACR) Annual Meeting in April of 2007, and as a result of this abstract submission, I have received a Scholar in Training Award from the AACR.

Additionally, the manuscript that has been produced from data analysis performed on existing breast cancer survival data is entitled, 'The Effect of Pre-Diagnostic Alcohol Consumption on Survival after Breast Cancer in Young Women.' The analysis is currently complete and the manuscript is being reviewed by co-authors. The next analysis I plan to undertake within this task of conducting data analysis on existing breast cancer data will involve investigating the role of oral contraceptive use and survival after breast cancer.

Nearly all tasks were completed as set out in the statement of work in the training grant application. Of those tasks that were not completed as originally anticipated, all had acceptable modifications substituted for the planned work. Firstly, the task of resequencing AKR1C1 to identify tagSNPs was modified due to the availability of existing, quality tagSNPs data for the region including AKR1C1 by the Hap Map project. The benefits from this modification allowed us to choose tagSNPs for the region encompassing AKR1C1 and AKR1C2 as a unit, and represented an improvement not only to the efficiency of the project but also to the scientific value of the investigation by characterizing jointly the AKR1C1 and AKR1C2 genes so that any shared structural elements were considered in the selection of tagSNPs.

Secondly, the plan to take a course entitled, 'Teaching and Mentoring,' and serve as a teaching assistant for the Introduction to Human Genetics, was replaced by the opportunity to teach a graduate level course in Biostatistics. During the course, I sought informal mentoring with instructors on topics including the incorporation of teaching methods for students of different learning styles and backgrounds. Benefits of this modification included gaining the experience of being the instructor for a course (rather than the teaching assistant), and hiring and training a teaching assistant.

In addition to the tasks listed in table 1, during this year I have met with the statistical geneticist on my doctoral dissertation committee, Chris Carlson, for biweekly meetings as suggested in the reviewers' comments, to discuss such topics as tagSNP selection, linkage disequilibrium, etc. Also, I have completed my doctoral general exams, and thus have progressed to the status of doctoral candidate. Additionally, I have attended FHCRC and UW seminars specific to breast cancer, as suggested in the

reviewers' comments, including but not limited to the Epidemiology of Breast Cancer seminar by Christopher Li and a lecture entitled, "Breast Cancer Follow-up Care," by Eva Grunfeld. I also plan to attend lectures focusing on breast cancer at the upcoming AACR Annual Meeting.

Key Research Accomplishments

1. Samples preparation for laboratory analysis, including DNA extraction and sample shipment
2. Completed grant applications for support of dissertation projects
3. Obtained IRB approval for dissertation research
4. Completed data analysis and manuscript preparation of existing breast cancer data
5. Completed data analysis on teaching strategies and presented findings at Teaching Symposium
6. Completed available academic courses
7. Completed the teaching of a course
8. Completed Doctoral general exam

Reportable Outcomes

Poster Presentation, AACR Annual Meeting 2007: 'Genetic polymorphisms in the estrogen metabolism pathway as modifiers of the effect of hormone therapy in breast cancer risk,' Kerryn W. Reding, Chu Chen, Christopher I. Lee, Christopher S. Carlson, Jasmine Wilkerson, Frederico M. Farin, Janet R. Daling, and Kathleen E. Malone

Manuscript, currently being reviewed by co-authors: 'The Effect of Pre-Diagnostic Alcohol Consumption on Survival After Breast Cancer in Young Women,' Kerryn W. Reding, Janet R. Daling, Cecilia A. O'Brien, David R. Doody, Peggy L. Porter, and Kathleen E. Malone

Manuscript, in preparation: 'Using Formative Assessments as a Student-Centered Approach to Improve the Implementation of Problem-Based Learning Modules,' Kerryn W. Reding, Laurence Wechsler, Thomas Koepsell, Deborah Hatch

Progression to Doctoral Candidate

Conclusion

In conclusion, I have made substantial progress towards the completion of my doctoral degree by completing the tasks set forth in year 1 of the BCRP Pre-doctoral training grant, and I am on track to complete the tasks in the subsequent years of this grant.

References

None.

Appendix 1
Supporting Documents

Table 1. Status of tasks outlined in the Statement of Work

TASK	STATUS
Task 1: Preparation for Lab Work (Months 1-4)	
a. Obtain Institutional Review Board approval	Completed
b. Identify and prepare blood samples for DNA extraction (sample size (n) =2362)	Completed
i. place samples in random order, intermixing cases and controls along with 10% quality control samples	Completed
c. Coordinate the delivery/shipping of extracted DNA to CEEH and TGen	Completed
d. Identify tagSNPs for AKR1C1 based on resequencing data (n = 24)	Completed; modified task[1]
e. Choose tagSNPs for AKR1C2, AKR1C3, SRD5A1, SRD5A2, PGR (SNPs already chosen for CYP1B1, COMT, and GSTs)	Completed
Task 2: Coursework and Training-related Work (Months 1-12)	
a. Complete courses:	
i. Gene Structure and Function	Not Completed[2]
ii. Advanced Genetics of Human Diseases	Completed
iii. Statistical Methods in Genetic Epidemiology	Completed
iv. Teaching and Mentoring	Modified task[3]
b. Prepare additional grants to support dissertation research	Completed
c. Conduct data analysis on existing breast cancer data	Ongoing[4]
d. Serve as Lead teaching assistant for Introduction to Epidemiology	Completed
e. Conduct research on active learning techniques in Introduction to Epidemiology	Ongoing[5]

TASK	STATUS of FUTURE TASKS
Task 3: Project Oversight of Genotyping of Samples (Months 5-24)	
a. Monitor progress of assay development and implementation	Ongoing task
b. Perform data management and project oversight	Ongoing task
c. Perform independent quality assurance of 10% of samples at FHCRC (n = 237)	Not Completed
d. Apply for and obtain IRB renewal	Ongoing task
Task 4: Training-related Work (Months 13-36)	
a. Present research findings on active learning at the UW Scholarship of Teaching and Learning Symposium	Completed
b. Conduct data analysis on existing data related to breast cancer etiology	Planned for months 16-36
c. Serve as a teaching assistant for Introduction to Genetics	Modified Task[3]
Task 5: Data Analysis and Report Writing (Months 25-36)	
a. Perform data cleaning and coding of variables	Ongoing; planned as future task[6]
b. Perform statistical analysis for each Specific Aim	Ongoing and future task
i. Impute haplotypes using PHASE v.2 software	Future
ii. Using STATA v.8, perform logistic regression analysis	Ongoing and future task
iii. Using STATA v.8, perform polytomous regression analysis	Future
c. Prepare manuscripts	Planned as future task[7]
d. Present results at DOD Era of Hope conference	Planned for Summer 2008

[1] TagSNPs were instead chosen from the Genome Variation Server, http://gvs.gs.washington.edu/GVS, as sponsored by Seattle SNPs

[2] GENOME 371: Gene Structure and Function was not offered in this academic calendar year and will be considered in the future

[3] Instead I taught an Introduction to Biostatistics to graduate students

[4] This work yielded a manuscript, entitled 'The Effect of Pre-Diagnostic Alcohol Consumption on Survival After Breast Cancer in Young Women,' that is currently being reviewed by co-authors.
[5] This work yielded a manuscript entitled, 'Using Formative Assessments as a Student-Centered Approach to Improve the Implementation of Problem-Based Learning Modules,' that is currently encorporating authors' comments
[6]The genotyping project from the CEEH for CYP1B1, COMT, and the GST genes has been completed; thus analyses have begun on this project. Analyses for the genetic variation in the progesterone pathway has not begun as genotyping for this project has not been completed.
[7] A poster investigating genetic variation in the estrogen pathway has been accepted for presentation at the AACR Annual Meeting in April of 2007.